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RESEARCH

SPRAY-DRIED LAMIVUDINE MICROSPHERES

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ABSTRACT

The objective of present study was to develop controlled release lamivudine microspheres using spray drying encapsulation technique to increase the efficacy of anti retroviral drug, lamivudine against HIV infections and decrease its gastric unwanted effects. Low and high molecular weight chitosan and hydroxypropyl methylcellulose (HPMC) in different drug-polymer ratios were used for the preparation of microspheres. A 3^2 factorial experimental design was employed to explore the effect of polymer type (X_1) and drug percentage (X_2) on the release rate of the drug from the microspheres. The yield value (Y_1), encapsulation efficiency (Y_2), particle size (Y_3) and the release efficiency percentage (Y_4) were selected as the dependent variables. Drug release from microspheres was compared with the release behavior of commercially available formulation Lamidine®. The best encapsulation efficiencies were obtained when chitosan of low molecular weight (CL) or HPMC were used for microencapsulation. Tissue Culture Infective Dose that causes CPE for 50 % of cells (TCID50) was calculated using Reed-Muench formula. TCID50 of the prepared formulae was higher than TCID50 of Control after 5 hours. Marked retardation of lamivudine release from its prepared microspheres indicates the success of controlling drug release over 5 hours.

1. INTRODUCTION

Lamivudine is a nucleoside reverse transcriptase inhibitor structurally related to cytosine with activity against retroviruses including HIV (Merril *et al*. 1995). It is used, usually with other antiretroviral, for combination therapy of HIV infection. It is also used for the treatment of chronic hepatitis B. Lamivudine is rapidly absorbed after oral doses and peak plasma concentrations are achieved in about 1 hour. Bioavailability is between 80 and 87 % (Sweetman 2007). An elimination half-life of 5 to 7 hours has been reported for a single dose (Betty 2000, Anthony and Clifford 2001) thus necessitating frequent administration to maintain constant therapeutic drug levels (Himadrisen and Surva Kumar 2005).
Much of the research effort in developing novel drug delivery systems has been focused on oral controlled-release dosage forms. Among them, multiple-unit dosage forms, such as microspheres, have gained much popularity for different reasons when compared to non-disintegrating single-unit dosage forms. They distribute more uniformly in the gastrointestinal tract, resulting in more reproducible release profiles, predictable gastric emptying, uniform drug absorption and reduced local irritation, minimized risk of dose dumping and avoid the unwanted intestinal retention of the polymeric material (Cuna *et al* .2000).

Spray drying is a one-step straight forward method for preparing microparticles and is preferable over other multi-step techniques such as solvent evaporation, solvent extraction and phase separation (Moretti *et al*. 2001) due to the fact that these conventional microencapsulation methods generally based on organic solvents. The use of organic solvents incurs risks of toxicity and explosions (Takeuchi *et al*. 1989).

Different microcapsules for sustained release formulations have been prepared by this method (Moretti 2001, Palmieri 2001). Microparticles prepared by spray drying can be used as oral dosage forms (dry powders, granules or agglomerates) (Wang 2002), targeted systems to organs and tissues, long acting parenteral biodegradable systems (He *et al.* 1999) and nasal powders (Illum *et al.*1994). Therefore, spray drying is an ideal process where the end-product must comply with precise quality standards regarding particle size distribution, residual moisture content, bulk density and particle shape.

Controlled release formulations of Lamivudine can overcome some of many problems including poor patient compliance and adverse side effects due to frequent dosing. Thus, the aim of this study was to prepare controlled-release microspheres of freely water soluble drug lamivudine adopting the spray drying encapsulation technique using chitosan or HPMC. The effect of different formulation and process variables were evaluated using experimental factorial design. Achieving this goal will enable us to reduce the administered dose, which is advantageous, taking in consideration the adverse effects of the drug and the cost-treatment relationship.

2. MATERIALS AND METHODS

2.1. Materials

Lamivudine USP was kindly supplied from Borg Pharmaceutical Ind., Hetero Drugs Limited, India. Disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from El-Nasr Pharmaceutical Chemicals Company, Egypt. Absolute ethyl alcohol, Chitosan (High molecular weight), Brookfield viscosity 800.000 cps and Chitosan (Low molecular weight), Brookfield viscosity 20 cps; were purchased from Sigma chemical Company; Germany. Hydroxypropyl methylcellulose-K100; was purchased from Fluka, Germany. All other chemicals and reagents were of the highest purity grade commercially available.

2.2. Methods

2.2.1. Preparation of Lamivudine Microspheres

Lamivudine microspheres were prepared by spray-drying using a Büchi Fluidized spray dryer (Model FSD-6.3; Büchi, Switzerland) with a standard 0.5 mm nozzle. When the liquid was fed to the nozzle with a peristaltic pump, atomization occurred by the force of the compressed air, disrupting the liquid into small droplets. The droplets, together with hot air, were blown into the chamber where the solvent in the droplets was evaporated and discharged out through an exhaust tube. The dry product was then collected in a collection bottle (Gavin *et al*. 2006).

The process conditions of the spray drying process were: inlet temperature 140°C; outlet temperature 95°C; Pump rate of 3ml/min and spray pressure about 2atm.

2.2.1.1. Preparation of lamivudine microspheres using chitosan

Two types of chitosan at 1 % (w/v) concentration namely high molecular weight and low molecular weight were solubilized in aqueous acetic acid solution (1% w/v). To obtain microspheres with different theoretical drug loading, amount was dissolved at different concentrations in 96% ethanol and mixed with chitosan in a 1:1 (v/v) ratio using magnetic stirrer at 8000 rpm for 10 min (Filipović-Grčić *et al*. 2003, Desai and Park 2005). The mixtures were subjected to spray-drying as described above. Blank microspheres were also prepared.

2.2.1.2. Preparation of lamivudine microspheres using HPMC

HPMC was dissolved in a mixture of 96% ethanol and water (2:3 v/v). The polymer concentration was 1 % (w/v). To obtain microspheres with different theoretical drug loadings, amount was dissolved at different concentrations in ethanol and mixed with HPMC in a 1:1 (v/v) (Filipović-Grčić *et al*. 2003). The mixtures were spray-dried under the conditions described above. Blank microspheres were also prepared.

2.2.2. Optimization of spray-dried lamivudine microspheres using a 3^2 factorial experimental design

Spray-dried lamivudine microspheres were prepared using a 3^2 factorial experimental design in order to investigate the joint influence of formulation variables and experimental conditions. 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The independent variables were polymer type (X_1) and drug percentage (X_2). The yield value (Y_1), encapsulation efficiency (Y_2), particle size (Y_3) and release efficiency percentage (Y_4) were selected as the dependent variables. Table I depicts the composition of the prepared microspheres and the results of yield value, encapsulation efficiency, particle size and release efficiency percentage.

2.2.3. Evaluation of the Prepared Microspheres

2.2.3.1. *Yields of Production*

The yield of production was calculated as the weight percentage of the final product after drying, with respect to the total amount of lamivudine and polymer used for preparation (Gohel *et al.* 2005).

$$\text{Percentage yield} = \frac{\text{weight of microspheres recovered x 100}}{\text{weight(drug + polymer)}} \quad \text{Eq.1}$$

2.2.3.2. *Encapsulation Efficiency (EE)*

Lamivudine chitosan microspheres (50 mg) were suspended in 15 ml of 0.1N HCl and then sonicated for about 20 min. The suspension was shaken for another 20 min for the complete extraction of the drug from the microspheres. The mixture was filtered through a 0.45µm membrane filter (Millipore). Drug content was determined by UV visible spectrophotometer at 279 nm (Gohel *et al.* 2005). The percent entrapment was calculated using the following equation (Parakash *et al*. 2007).

$$\text{Encapsulation Efficiency} = \frac{\text{Actual drug content x 100}}{\text{Theoretical drug content}} \quad \text{Eq.2}$$

The HPMC microspheres (50 mg) loaded with lamivudine were suspended in the mixture of 0.1N HCl and 96% ethanol (1:1, v/v; 15 ml) (Filipović-Grčić *et al*. 2003) then the same procedure was done as chitosan microspheres described above.

2.2.3.3. *Scanning Electron Microscopy*

The surface morphology of lamivudine microspheres prepared using spray drying were examined by the scanning electron microscope (Joel JXA-840A; Tokyo, Japan) following methodology described by Sheu *et al*. 1998. Microspheres were attached to SEM Stubs (10 mm) using a double-sided adhesive tape and then were made electrically conductive by coating, in a vacuum, with a thin layer of gold. The pictures were taken using SEM operated at 15 KV.

2.2.3.4. *Particle Size Distribution Analysis*

Particle size analysis was done in Mastersizer Ver.2.15 (Malvern Instruments Ltd., UK) equipment, following procedure described into the manual of the equipment. Microspheres were suspended in ethanol and sonicated 1.5 min during the analysis (in triplicates); the samples were maintained in constant agitation.

The particle size distribution was expressed in terms of SPAN factor determined as:

$$\text{SPAN} = \frac{d_{90} - d_{10}}{d_{50}} \quad \text{Eq.3}$$

Where d_{10}, d_{50} and d_{90} are the diameter sizes and the given percentage value is the percentage of particles smaller than that size. A high SPAN value indicates a wide distribution (Dubey and Parikh 2004).

2.2.3.5. *In-Vitro Release Studies Of Lamivudine from the Prepared Microshperes*

The *in-vitro* release of lamivudine from the prepared microspheres was carried out in 900 ml of Sorensen's phosphate buffer of pH 7.4 (Filipović-Grčić *et al*. 2003) at a temperature of 37 ± 0.5°C using the USP Dissolution Tester, Apparatus I (Rotating basket) (Parakash *et al*. 2007, Ravi *et al*. 2007) at 50 rpm. Aliquots each of 5 ml were withdrawn at specified time intervals and replaced by equal volumes of fresh medium. The samples withdrawn were then filtered through a Millipore filter of 0.45 µm and analyzed for lamivudine content by measuring the absorbance at λmax 270 nm using blank microspheres as a blank.

Release efficiency percentage after 5 hours (RE5 %) was considered as a basis for comparison of the release and was calculated based on the following equation (Khan 1975):

Release Efficiency (RE %) = $^{o}\int t$ y. dt / y100 t * 100 Eq.4

2.2.3.6. *Kinetic Analysis of the Release Data*

To determine the mechanism of release of lamivudine from its different microspheres formulae, the release data were analyzed using the linear regression according to:

Zero order (Wagner 1998): $C_t = C_\circ - Kt$,

The simplified Higuchi diffusion model (Higuchi 1961):

$Q/A = 2C_\circ (A/\pi)^{1/2} t^{1/2}$

And Korsemeyer-Peppas (Peppas 1985): $M_t/M_\infty = Kt^n$

Where M_t/M_∞ is the fraction of drug released at time t and k denotes the constant incorporating structural and geometrical characteristics of the drug/polymer system and the n is the diffusion exponent related to the mechanism of the drug release. For non Fickian (anomalous) release from spheres, the n value falls between 0.43 and 0.85 (where release is controlled by a combination of diffusion and polymer relaxation) while for Fickian (case I) diffusion, $n \leq 0.43$ ($t^{1/2}$ dependence) and for Zero-order release (Case II transport), n = 0.85 where the drug release rate is independent of time and involves polymer relaxation and chain disentanglement**.** It is important to note that for determination of the exponent *n,* only the initial portion of the release curve ($M_t/M_\infty \leq 0.6$) must be used (Peppas 1985).

The value of K and n were estimated by linear regression of log (M_t/M_∞) on log (t) where log K is the intercept and n is the slope of the straight line.

Log M_t/M_∞ =log K + n log t

The correlation coefficient (R^2) was determined in each case. The large value of the coefficient of determination (R^2) indicated a superiority of the dissolution profile fitting to mathematical equations.

2.2.4. Preparation of Lamivudine Capsules:

Based on the results obtained in the present study, it could be recommended the choice of H2, composed of lamivudine and HPMC in the ratio of 1:5 drug : polymer, for further investigations. This formula presented the most optimum results, highest yield (44.71 %), with highest encapsulation efficiency (99.70 %) and *in-vitro* release profiles as reflected by the decrease in the release efficiency based on 300 min (R.E %= 42.18%).

A sample of the selected microsphere (H2) equivalent to 150 mg lamivudine (according to the pre-calculated drug content) was accurately weighed and filled in hard gelatin capsules.

2.2.5. Evaluation of the Prepared Capsules:

The prepared capsules and the commercially available Lamidine® tablets were subjected to:

2.2.5.1. *In-vitro Release Studies*

The *in-vitro* release of commercially available Lamidine® tablets and the prepared capsules was carried using the same procedure as previously mentioned.

2.2.5.2. *Antiviral Evaluation of Lamivudine from its Prepared Capsules*

The experiment was carried out in 200 ml phosphate buffer of pH 7.4 at a temperature of 37 ± 0.5°C using the Julabo Thermostatic Shaker, where each of the prepared capsules and commercially available Lamidine® tablet were placed inside a semi permeable membrane and added to the medium at time zero. After 1 hour a sample of 20 ml was drawn and filtered through a Millipore filter of 0.45µm. Then the medium was discarded and replaced with fresh 200 ml phosphate buffer. The same procedure was repeated after 3 and 5 hours.

2.2.5.3. *Determination of the cytopathic effect (CPE) of the solutions*

The assays were performed using 96-well flat bottom tissue culture plates (gamma sterilized, tissue culture treated, TPP). Initially all wells were supplemented with Vero cell line CCL81, maintained on E-199 medium supplemented with 10 % activated foetal bovine serum, 100 µg/ml penicillin and 10 µg/ml streptomycin. Then plates were incubated at 37°C in double door incubator until confluent sheet of cells was microscopically detected using inverted microscope.

About 100 µl of two fold serial dilutions of all tested solutions were added into the wells in quadruplicate. All plates were then incubated at 37 °C in a 5% CO_2 atmosphere. After 24 h, the cytopathic effect (CPE) was determined by two ways:

1) Microscopically, using the inverted microscope.

2) Safranine dye method where safranine was added and the cultures were incubated for one hour and then washed using PBS (Phosphate-Buffered Saline) many times to remove excess dye and then the stained wells were determined to estimate the safe concentrations of the extracts which didn't affect the integrity of cell monolayers.

2.2.5.4. *Antiviral assay*

The assays were performed using 96-well flat bottom tissue culture plates (gamma sterilized, tissue culture treated, TPP). The safe concentration of each solution (where no CPE effect) was added to the monolayers of Vero cell line CCL81 maintained in E-199 medium, supplemented with 10 % activated foetal bovine serum, 100 µg/ml penicillin and 10 µg/ml streptomycin. All plates were incubated at 37°C in a 5% CO_2 atmosphere using CO_2 incubator (Double door incubator). After 24 h, the cell monolayers integrity was checked microscopically. Then the medium was washed off and the stable monolayers were then treated with the 10 fold serially-diluted (Vesicular stomatitis virus) with initial titre of 107.8/0.1 ml. The plates were incubated at 37°C in a 5% CO_2 atmosphere. The CPE was checked at different time intervals (24, 48

and 72 h). The CPE was checked microscopically. Control was conducted using reference market product tablet. All experiments were conducted in five replicates under aseptic conditions.

According the recorded CPE from different dilutions the infectivity and Tissue Culture Infective Dose that cause CPE for 50 % of cells (TCID50) was calculated using Reed-Muench formula (Reed and Muench 1983).

3. RESULTS

The spray drying method used appeared to be a suitable and simple technique to prepare chitosan or HPMC microspheres loaded with lamivudine. Other techniques such as emulsification /solvent evaporation involve different steps and the use of surfactant to stabilize the emulsion (Gavin *et al*. 2006).

3.1. Evaluation of the Prepared Microspheres:

In the present study two types of chitosan, with different molecular weight, and HPMC were used for the preparation of microspheres. The amount of lamivudine varied among the preparations while the amount of the polymer used, chitosan or HPMC, was kept constant.

3.1.1. *Yield of production*

For chitosan microspheres loaded with lamivudine

Yield of production ranged between 22.43 and 38.60%, the microspheres prepared using chitosan low molecular weight showed the highest values. The yield value of the chitosan microspheres is reported in table (I).

For HPMC microspheres loaded with lamivudine

Yield of production ranged between 40.00 and 44.71 %. The highest yield value (44.71 %) was obtained for H2 microspheres. The yield value of the HPMC microspheres is reported in table (I).

3.1.2. *Encapsulation Efficiency*

For chitosan microspheres loaded with lamivudine

The encapsulation efficiency ranged between 60.00 and 98.97 % as shown in table (I).

For HPMC microspheres loaded with lamivudine

The encapsulation efficiency ranged between 82.59 and 99.70% as shown in table (I). The average degree of drug encapsulation into microspheres improved with respect to previous CH chitosan microspheres, while it was similar to the encapsulation efficiency of CL chitosan microspheres. The highest enapsulation efficiency (99.70%) was obtained for H2 microspheres.

3.1.3. *Scanning Electron Microscopy*

The surface morphology of the prepared chitosan microspheres was investigated by Scanning Electron Microscopy (Fig. 1). Lamivudine loaded chitosan microspheres prepared by spray drying appeared to be spherical, smooth, homogeneously distributed without evidence of collapsed particle. Chitosan based microspheres showed smooth surface and exhibited regular spherical geometry. The SEM photograph did not show any aggregation of microspheres and there was no grafting of polymer in chitosan microspheres.

The surface morphology of the HPMC based microspheres appeared to be spherical, smooth and with good morphological characteristics (Fig. 2). The same observation was recorded when spray- dried carbamazepine-loaded chitosan and HPMC microspheres were prepared (Filipović-Grčić *et al.* 2003).

3.1.4*. Particle Size Distribution*

For chitosan microspheres loaded with lamivudine

The prepared microspheres have spherical diameter ranging from 3.12 to 4.85 μm (Table I).

Neither chitosan molecular weight nor drug loading of the microspheres influenced particle size characteristics. This fact was already reported for chitosan microspheres prepared by other methods (Berthold *et al.* 1996, Genta *et al.* 1998, Filipović-Grčić *et al.* 2003) even though He et al, 1999 found influence of chitosan molecular weight on microspheres size (He *et al.* 1999).

For HPMC microspheres loaded with lamivudine

The prepared microspheres have spherical diameter ranging from 3.66 to 4.32 μm (Table I). Drug loading of the microspheres didn't influence particle size characteristics.

3.1.5. *In -Vitro release studies*

For chitosan microspheres loaded with lamivudine

The release profiles of lamivudine from the chitosan microspheres are shown in figures (3 and 4). The effect of drug loading on the release of lamivudine from chitosan based microspheres was reflected on the values of the release efficiency (RE %), where (% RE5) of CH1 and CL1 with low lamivudine loading was 47.37% and 57.32 % respectively. The release efficiency (% RE5) of CH3 and CL3 with high lamivudine loading was 78.43 % and 70.54 % respectively. Results are shown in table (I).

For HPMC microspheres loaded with lamivudine

Figure (5) represent the effect of HPMC content on lamivudine release from the microspheres. It was found that increasing the HPMC content in the matrix decreased the amount of lamivudine released. Only 58.59% of lamivudine was released from HPMC microspheres with a drug loading of 9.09% (w/w) within 5 hours, while 90.31% was released in the same period with a drug loading of 33.33 % (w/w).

3.1.6. *Kinetic Analysis of the Release Data*

The kinetic analysis of the *in-vitro* release data of lamivudine from chitosan and HPMC microspheres are tabulated in table (II). According to correlation coefficient (R2), the *in-vitro* release data were in favor of diffusion release kinetics (for formulae CH1, CH2, CL1, CL2, CL3, H2 and H3) and Koresmeyer-Peppas release kinetics (for formulae CH3 and H1).The values of n were ≤ 0.43 indicating Fickian (case I) diffusion transport for most formulae (except for formulae H1 and H2).

3.2. Evaluation of the Prepared Capsules

3.2.1. *In-vitro release studies*

The release profiles of lamivudine from the prepared capsule formula as well as of the market product tablet in Sorensen's phosphate buffer of pH 7.4 were studied. The results were graphically represented in Figure (6).
It was evident that the prepared formulae effectively controlled the release of lamivudine compared to immediate release of lamivudine from the market product. The percent of drug released was found to be 87.00 % after 10 min from the commercial tablets. In case of the prepared capsules a noticeable and gradual control of release behavior of lamivudine could be observed during the whole release run (65.00% of lamivudine was released from H2 after 5 hours).

3.2.2. *Determination of the cytopathic effect (CPE) of the solutions*

The cytopathic effect (CPE) of the selected capsules formula as well as of the market product tablet in Sorensen's phosphate buffer of pH 7.4 was studied. The results were graphically represented in figure (7).

3.3. Antiviral assay

According the recorded CPE from different dilutions the infectivity and Tissue Culture Infective Dose that cause CPE for 50 % of cells (TCID50) was calculated using Reed-Muench formula. The results are graphically represented in figure (8).
It was evident that the prepared formula effectively controlled the release of lamivudine compared to the market product. TCID50 of the prepared formula was higher than TCID50 of Control after 5 hours.

4. DISCUSSION

4.1. *Yield of production*

The relatively low values of yield of production could be due to both the small volumes of feed solution and the structure of the spray dryer apparatus, which was not supplied with a trap to recover the smaller and lighter particles which exhausted by the aspirator, as already pointed out (Giunchedi *et al*. 2000) [34]. The yield value of the chitosan microspheres is shown in Table (I).

Table I. Experimental runs, independent variables and measured responses of the 3^2 factorial design.

Formula	X_1: Polymer type	X_2: Drug percentage	Y_1: Yield value	Y_2: Encapsulation efficiency	Y_3: Particle size	Y_4: Release efficiency percentage
CH1	CH	9.09	23.51 ± 2.50	66.21 ± 0.97	4.77 ± 0.99	47.37 ± 0.34
CH2	CH	16.67	22.43 ± 4.21	60.00 ± 2.40	4.85 ± 1.18	56.02 ± 0.34
CH3	CH	33.33	25.18 ± 3.95	89.40 ± 1.50	4.66 ± 0.42	78.43 ± 0.19
CL1	CL	9.09	35.80 ± 2.30	98.97 ± 0.74	4.84 ± 0.91	57.32 ± 0.26
CL2	CL	16.67	37.50 ± 1.40	70.70 ± 0.53	4.81 ± 1.03	64.94 ± 0.28
CL3	CL	33.33	38.60 ± 2.10	83.50 ± 2.70	3.12 ± 0.75	70.54 ± 0.28
H1	HPMC	9.09	40.00 ± 1.92	82.59 ± 2.50	3.88 ± 0.76	38.36 ± 0.34
H2	HPMC	16.67	44.71 ± 2.35	99.70 ± 0.30	4.32 ± 0.61	42.18 ± 0.27
H3	HPMC	33.33	42.17 ± 1.67	84.62 ± 3.20	3.66 ± 1.01	62.75 ± 0.27

4.2. *Encapsulation Efficiency*

Comparing the encapsulation efficiency obtained with the two types of chitosan used (CL and CH), the highest encapsulation efficiency (98.97%), was obtained when CL chitosan was used for encapsulation probably due to the highest degree of deacetylation of CL chitosan (87.40 %). Similar findings have already been reported by Genta *et al* 1998, Filipović-Grčić. *et al.* 2003) who found that the best encapsulation efficiencies were obtained when chitosan of low molecular weight (CL) or HPMC were used for the microencapsulation of carbamazepine in an attempt to modify its release profile.

4.3. *In -Vitro release studies*

4.3.1. For chitosan microspheres loaded with lamivudine

When microspheres of hydrophilic polymers are immersed in water, they swell and form a gel diffusion layer that hinders the outward transport of the drug within the matrix, hence producing a controlled-release effect (Lim *et al*. 2000). As the amount of polymer increases, the thickness of the hydrogel layer increases as well, and the drug diffusion is more retarded. That could explain the slower release of water soluble lamivudine from the microspheres with low drug loading.
Figures (3 and 4) also show an initial burst effect of lamivudine release from all batches of microspheres. This fact, already noticed by He et al (1999), was most likely due to the hydrophilic character of the chitosan and to the small dimensions of the microspheres. The initial rapid release has been reported not only to occur with water soluble drugs but also with some less water soluble drugs, such as dexamethasone (Pavanetto *et al*. 1994) and nicardipine (Conte *et al*. 1994).
It must be pointed out that the initial rapid drug release may have a functional use in providing an initial dose during the drug delivery, minimizing any lag period. The microspheres with the lowest drug loading presented the lowest burst effect (Genta *et al* 1998).

4.3.2. For HPMC microspheres loaded with lamivudine

The microspheres with high drug content were expected to be more porous than those with low drug content, which might facilitate the release of residual drug from microspheres(Wan *et al*. 1994). The effect of drug loading on the release of lamivudine from chitosan based microspheres was reflected in the values of the release efficiency (RE %), where (% RE5) of H1 with low lamivudine loading was 38.36 % compared to 62.75% (RE5 %f H3) with high lamivudine loading. Results are shown in table (I).

4.4. *Analysis of factorial design*

The factorial design, a commonly used statistical approach for planning and optimization of experimental series, was used. The used design comprises a full 3^2 factorial design. The experimental runs, with independent variables and the measured responses were statistically analyzed through Design-Expert ®Software as shown in (table I). ANOVA test was performed to evaluate the significance of the tested factors on the yield value, encapsulation efficiency, particle size and release efficiency.

Results showed that the polymer type showed a significant effect on the yield value and the release efficiency % of the spray-dried lamivudine microspheres *(p* = 0.004, 0.041) respectively as shown in figures (10, 11). Results also showed

that changing the drug:polymer ratio showed a significant effect on the release efficiency % of the spray-dried lamivudine microspheres *(p* = 0.015) as shown in figure (12).

On the other hand, the polymer type has a non significant effect on neither the encapsulation efficiency nor the particle size (*p* = 0.441, 0.243) respectively. Results also showed that changing the drug:polymer ratio has a non significant effect on neither the yield value, the encapsulation efficiency nor the particle size of the spray-dried lamivudine microspheres (*p* = 0.319, 0.776, 0.198) respectively.

4.5. Evaluation of the Prepared Capsules:

4.5.1. *In-vitro* Release Studies*:*

The release profile of lamivudine from the prepared capsule formula as well as of the market product tablet in Sorensen's phosphate buffer of pH 7.4 was studied. The results were graphically represented in figure (6).

It was evident that the prepared formula (H2) effectively controlled the release of lamivudine compared to the market product. 96.65% of lamivudine was released from the market product in 90 min.

In case of H2 a noticeable and gradual control of release behavior of lamivudine could be observed during the whole release run (65.00 % of lamivudine is released from H2 after 5 hours).

4.5.2. Antiviral assay:

It was evident that the prepared formula effectively controlled the release of Lamivudine compared to the market product. TCID50 of the prepared formula was higher than TCID50 of Control after 5 hours. This means that the infectivity of the virus decreased so the cells were protected due to the presence of the drug.

TCID50 of the market product was equal to that of the control. This means absence of drug after 3 and 5 hours.

In the case of the controlled release formulation H2, it is clear that the percent reduction of the viral infectivity was found to be 80 % and 90 % after 3 and 5 hours respectively compared to an absence of the infectivity in case of the immediate release tablets.

Table 2. Kinetic analysis of the in vitro release data of lamivudine from microspheres prepared using spray drying

Formulae	Zero			Diffusion			Peppas			Release Order	K	n
	Slope	Intercept	R2	Slope	Intercept	R2	Slope	Intercept	R2			
CH1	0.189	15.780	0.981	3.684	5.500	0.993	0.367	0.863	0.992	Diffusion	7.310	0.367
CH2	0.263	21.035	0.932	4.083	11.264	0.959	0.307	1.027	0.934	Diffusion	10.630	0.307
CH3	0.459	33.631	0.935	5.249	20.060	0.980	0.330	1.260	0.99	Peppas	18.220	0.330
CL1	0.254	23.070	0.972	4.050	10.600	0.996	0.343	1.040	0.991	Diffusion	10.910	0.343
CL2	0.404	26.510	0.963	5.270	11.490	0.991	0.361	1.080	0.986	Diffusion	11.940	0.361
CL3	0.488	29.750	0.984	5.490	15.770	0.999	0.315	1.210	0.996	Diffusion	15.880	0.315
H1	9.811	10.402	0.99	27.795	0.013	0.989	0.468	1.381	0.991	Peppas	24.097	0.468
H2	13.100	7.531	0.966	32.170	-3.272	0.995	0.646	1.432	0.988	Diffusion	27.087	0.646
H3	0.298	23.710	0.977	4.257	12.690	0.985	0.282	1.120	0.973	Diffusion	13.120	0.282

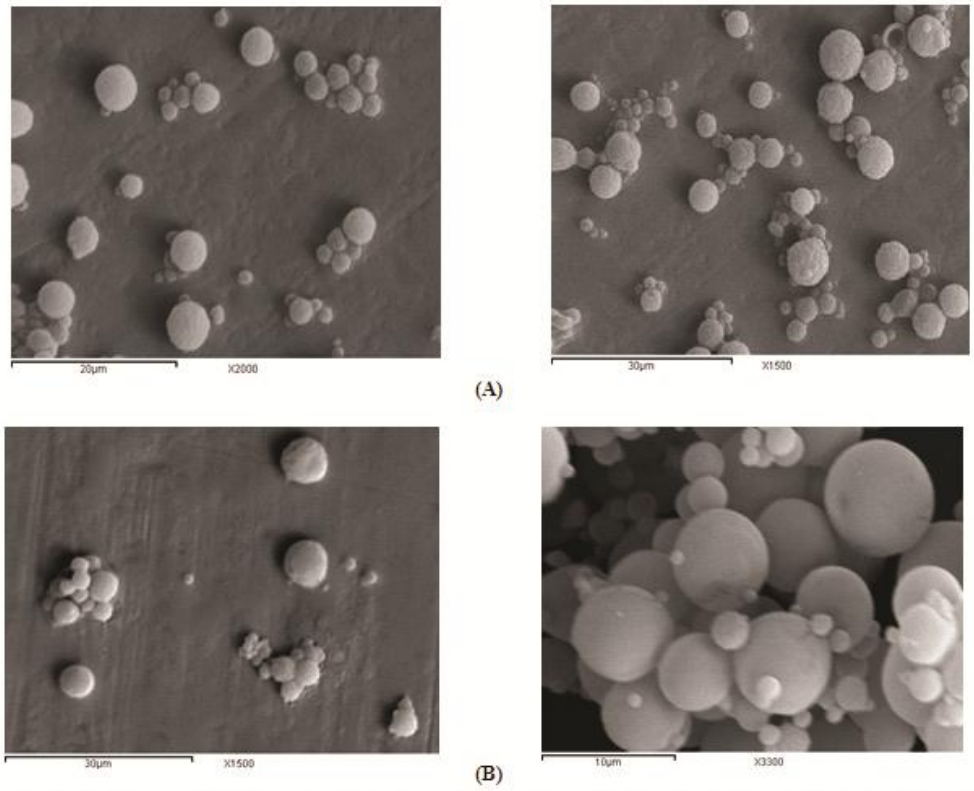


Fig. (1): Scanning electron micrographic photographs of plain chitosan microspheres (A) and Lamivudine loaded chitosan microspheres (B).

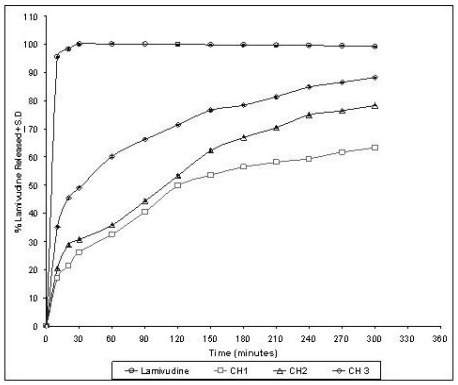


Fig. (3): *In-vitro* release profile of Lamivudine from microspheres prepared using high molecular weight chitosan in Sorensen's phosphate buffer of pH 7.4.

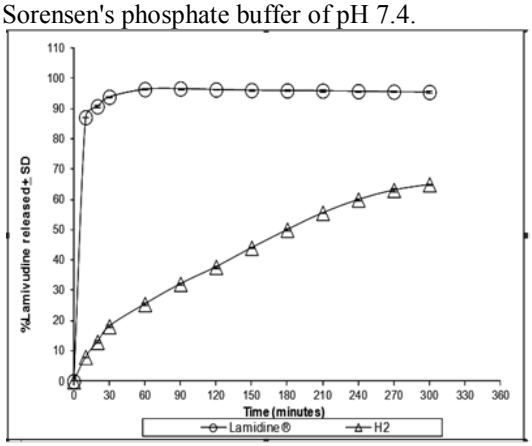


Fig. (5): *In-vitro* release profile of Lamivudine from microspheres prepared using HPMC in Sorensen's phosphate buffer of pH 7.4.

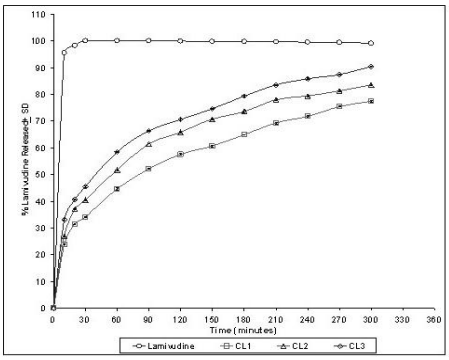


Fig. (4): *In-vitro* release profile of Lamivudine from microspheres prepared using low molecular weight chitosan in Sorensen's phosphate buffer of pH 7.4.

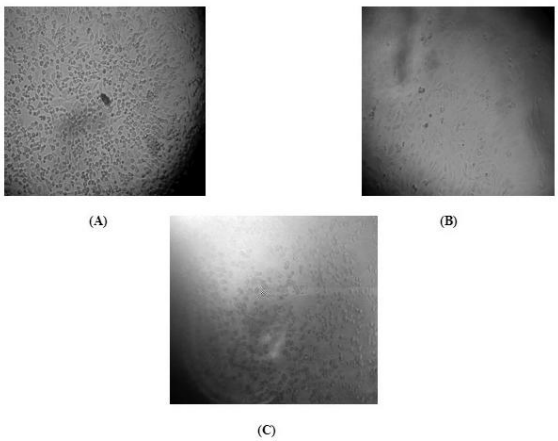
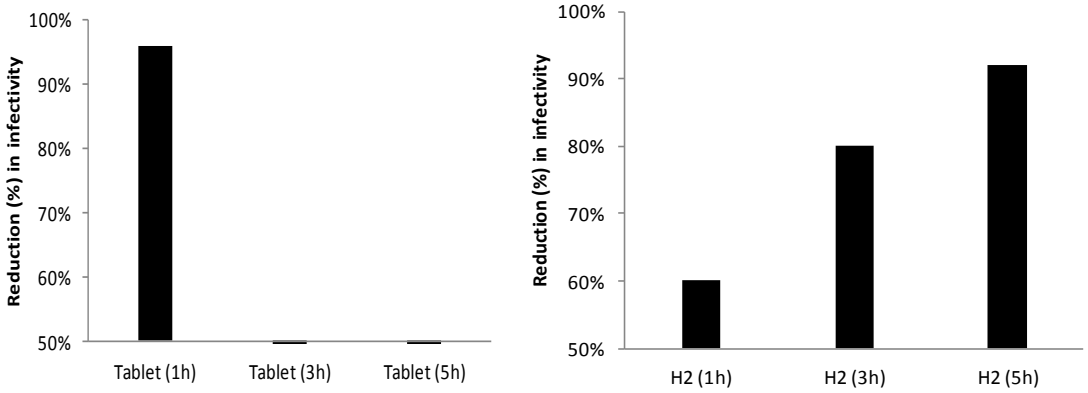


Fig.7 Determination of Cytopathic effect of Lamivudine from the prepared capsules formulae and the market product. (A) Cytopathic effect, (B) Non Cytopathic effect and (C) Intermediate Cytopathic effect.



(A) **(B)**

Fig. (8): The % reduction of the viral infectivity (TCID50) of the blank used compared to the reference Lamidine tablet (A) and the controlled release formula H2 (B).

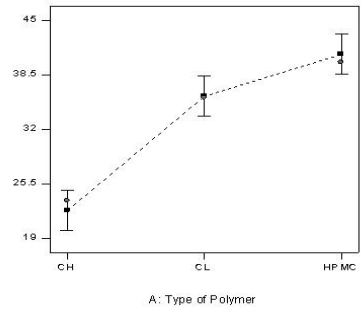


Fig. (9): Effect of Changing Polymer Type on the Yield value of the Spray Dried Lamivudine Microspheres.

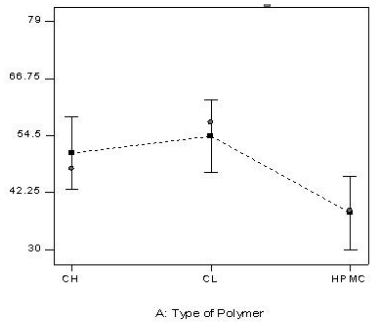


Fig. (10): Effect of Changing Polymer Type on the Release Efficiency % of the Spray Dried Lamivudine Microspheres.

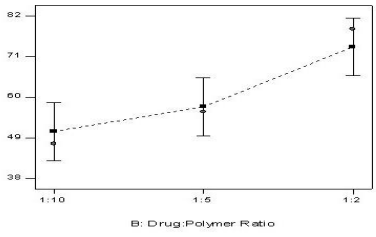


Fig. (11): Effect of Changing Drug:Polymer Ratio on the Release Efficiency % of the Spray Dried Lamivudine Microspheres.

CONCLUSION

This work confirms the feasibility of the spray drying for the preparation of multiparticulate systems to modify lamivudine release. Results indicate that lamivudine release from microspheres can be controlled by a proper choice of polymer type, polymer amount and spray drying operating conditions. Marked retardation of lamivudine release may hold promise for applications in anti retroviral drug delivery systems.

REFERENCES

1. Anthony S.F. and Clifford H.L. (2001) Human immunodeficiency virus (HIV) disease: AIDS and related disorders. In: *Harrisons Principles of Internal Medicine* (eds Braunwald E., Fauci A.S., Kasper D.L., Hauser S.L.,Longo D.L., Jameson J.L.), pp. 1852-1913. McGraw-Hill, New York.
2. Berthold A., Cremer K., Kreuter J. (1996) Preparation and characterisation of chitosan microspheres in drug carrier for prednisolone sodium phosphate as model for anti-inflammatory drugs. *J. Control. Rel*. **39**, 17-25.
3. Betty J.D. (2000) Human immunodeficiency virus (HIV)-antiretroviral therapy. In: *Textbook of Therapeutics: Drug and Disease Management* (eds Herfindal E.T. & Gouley D.R.), pp. 1555-1582. Lippincott Williams & Wilkins, Philadelphia Pa.
4. Conte U., Giunchedi P., Maggi L., Torre M.L. (1994) Spray dried albumin microspheres containing nicardipine. *Eur. J. Pharm. Biopharm*. **40**, 203-208.
5. Cuna M., Vila Jato J.L., Torres D. (2000) Controlled-release liquid suspensions based on ion-exchange particles entrapped within acrylic microcapsules. *Int. J. Pharm.* **2**,151–158.
6. Desai K.G.H., Park H.J. (2005) Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. *J. Microencapsul*. **22(2)**, 179-192.
7. Desai K.G.H., Park H.J. (2005) Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. *J. Microencapsul*. **22 (2)**, 179-192.
8. Dubey R.R., Parikh R.H. (2004) Studies of PLGA microspheres. *Pharm. Tech. Eur*. **16**, 23-34.
9. Filipović-Grčić J., Perissutti B., Moneghini M., Voinovich D., Martinac A., Jalšenjak I. (2003) Spray dried carbamazepine-loaded chitosan and HPMC microspheres: preparation and characterization. *J. Pharm. Pharmacol.* **55**, 921-931.
10. Gavin E., Hegge A.B., Rassu G., Sanna V., Testa C., Pirisino G., Karlsen J., Giunchedi P. (2006) Nasal administration of carbamazepine using chitosan microspheres, In-vitro/in-vivo studies. *Int. J. Pharm.* **307**, 9-15.
11. Genta I., Perugini P., Pavanetto F. (1998) Different molecular weight chitosan microspheres: influence on drug loading and drug release. *Drug Dev. Ind. Pharm*. **24**, 779-784.
12. Giunchedi P., Gavini E., Palmieri G.F., Bonacucina G. (2000) Tableted polylactide microspheres Prepared by a w/o emulsion-spray drying method. *J. Microencapsul*. **17**, 711-720.
13. Gohel M.C., Parikh R.K., Amin A.F., Surati A.K. (2005) Preparation and formulation optimization of sugar cross-linking gelatin microspheres of declofenac sodium. *Indian J. Pharm Sci*. **67**, 575-581.
14. He P., Davis S.S., Illum L. (1999) Chitosan microspheres prepared by spray drying. *Int. J. Pharm.* **187**, 53-65.
15. Higuchi T. (1961) Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci*. **50**, 874-875.
16. Himadrisen , Surva Kumar J. (2005) Long acting composition containing zidovudine and lamivudine. US patent publication US 20050175694A1, 11 August 2005.
17. Illum L., Farraj N.F., Davis S.S. (1994) Chitosan as novel nasal delivery system for peptide drugs. *Pharm. Res.* **11**, 1186-1189.
18. Khan K.A. (1975) The concept of dissolution efficiency. *J. Pharmacol*. **27**, 98-99.
19. Lim S.T., Martin G.P., Berry D.J., Brown M.B. (2000) Preparation and evaluation of the in vitro drug release properties and mucoadhesion of novel microspheres of hyaloranic acid and chitosan. *J. Control. Rel*. **66**, 281-292.
20. Merril D.P., Moonis M., Chou T.C .and Hirsch M.S. (1996) Lamivudine or stavudine in two - and three - drug combinations against human immunodeficiency virus type 1 replication in vitro. *J. Infect. Dis.* **173**, 355-364.
21. Moretti M.D.L, Gravini E., Juliano C., Pirisino G., Giunchedi P. (2001) Spray dried microspheres containing ketoprofen formulated into capsules and tablets. *J. Microencapsul.* **18**, 111-121.
22. Moretti M.D.L., Gravini E., Juliano C., Pirisino G., Giunchedi P. (2001) Spray dried microspheres containing ketoprofen formulated into capsules and tablets. *J. Microencapsul.* **18,** 111-121.
23. Palmieri G.F., Bonacucina G., Martino P., Martelli S. (2001) Spray-drying a method for microparticulate controlled release systems preparation: advantages and limits. I. Water soluble drugs. *Drug. Dev. Ind. Pharm.* **27**, 195-204.
24. Parakash K., Raju R.N., Shanta K.K., Lakshmi M.N. (2007) Preparation and characterization of lamivudine microcapsules using various cellulose polymers. *Tropical J. of Pharm. Res*. **6(4)**, 841-871.
25. Pavanetto F., Genta I., Giunchedi P., Conti B., Conte U. (1994) Spray dried albumin microspheres for the intra-articular delivery of dexamethasone. *J. Microencapsul*. **11**, 445-454.
26. Peppas N.A. (1985) Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta. Helv*. **60**, 110.
27. Ravi P.R., Ganga S., Saha R.N. (2007) Design and study of lamivudine oral controlled release tablets. *AAPS Pharm. Sci. Tech*. 8(4), 167–175..
28. Reed L.J., Muench H. (1983) A simple method of estimating fifty percent endpoints. *Am. J. Hyg*. **27**, 493- 497.
29. Sheu, T-Y. and Rosenberg M. (1998) Microstructure of microcapsules consisting of whey proteins and carbohydrates. *J. Food Sci*. **63(3)**, 491-494.
30. Sweetman S.C. (2007) *Martindale: The Complete Drug Reference.* Pharmaceutical Press.
31. Takeuchi H., Handa T., Kawashima Y. (1989) Controlled release thyophylline with acrylic polymers prepared by spray drying technique. *Drug. Dev. Ind. Pharm.* **15**, 1999-2016.

32. Wagner J.G. (1998) Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. *J. Pharm. Sci*. **58**, 1253-1257.
33. Wan L.S.C., Lim L.Y., Soh B.L. (1994) Drug release from chitosan beads. *STP Pharm. Sci*. **4**, 195-2000.
34. Wang F.J., Wang C.H. (2002) Effects of fabrication conditions on the characteristic of etanidazole spray-dried microspheres. *J. of Microencapsul.* **19**, 495-510.